IMPROVEMENT OF CONCRETE DURABILITY WITH THE AID OF BACTERIA

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Shortcomings of conventional surface coatings have drawn the attention to alternative treatments for the improvement of the durability of concrete. Promising results of an innovative biotechnology based on microbial carbonate precipitation have lead to research concerning the use of bacteria in or on concrete. In our research groups, first the criteria for the selection of calcium precipitating Bacillus strains were established. *Bacillus sphaericus* strains capable of the remediation of Euville limestone, by precipitating a dense and coherent calcium carbonate layer and concomitantly inducing a reduction of capillary water absorption, were characterised by a high urease activity, abundant EPS-production, a good biofilm production and a very negative ζ-potential.

This paper reports the effects of bacterial $CaCO_3$ precipitation on parameters affecting the transport processes and durability of concrete and mortar. Pure cultures of ureolytic bacteria were compared for their effectiveness depending on the presence and type of calcium source (calcium chloride or calcium acetate). Treatments were evaluated according their influence on transport processes such as water vapour permeability and water absorption. The durability of the treated substrate has been studied by measuring the resistance to carbonation and chloride ingress. Microbial calcite precipitation was quantified by X-ray diffraction (XRD) analysis and visualized by SEM. The results indicated the presence of a newly formed layer on the surface of the mortar specimens, consisting mainly of calcite. Bacterial deposition of a layer of calcite on the surface of the specimens resulted in a decrease of capillary water uptake and permeability towards gas. This bacterial treatment resulted in a limited change of the chromatic aspect of mortar and concrete surfaces.

Keywords: concrete, calcium carbonate precipitation, bacteria, durability, permeability

1 Introduction

Considerable research on carbonate precipitation by bacteria has been performed using ureolytic bacteria [1-3]. These bacteria are able to influence the precipitation of calcium carbonate by the production of a urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase of the pH and carbonate concentration in the bacterial environment [3]. Precipitation of calcium carbonate crystals occurs by heterogeneous nucleation on bacterial cell walls once supersaturation is achieved.

The fact that hydrolysis of urea is a straightforward common microbial process and that a wide variety of microorganisms produce the urease enzyme makes it ideally suited for biotechnological applications [4].
Research has indicated that a concrete which is low in permeation properties lasts longer without exhibiting signs of distress and deterioration [5]. Therefore, the permeation properties have been used principally for the comparison of the effectiveness of different surface treatments enhancing the durability of concrete. Microbial carbonate precipitation (biodeposition) decreases the permeation properties of mortar and concrete, as was shown in our previous study. The deposition of a layer of calcium carbonate on the surface of the mortar specimens resulted in a decrease of water absorption and gas permeability [6, 7].
In the current study, the effectiveness of the biodeposition treatment was investigated by evaluating the resistance of mortar specimens towards degradation processes. Mortar cubes of varying porosity (water-cement ratio), were treated and subjected to accelerated carbonation and chloride migration.

2 Materials and method

2.1 Mortar mixture proportions and dimensions of specimens

The mortar samples were made using a normal portland cement (CEM I 52.5 N) content of 350 kg/m³, sand 0/4 content of 1050 kg/m³ and water-cement ratios of 0.5, 0.6 and 0.7. Average compressive strength of each mixture was obtained from the measurements (according the Belgian standard NBN B15-220) on three cubes (100 x 100 x 100 mm³) after 28 days of water curing and amounted to 50.3 ± 3.96 MPa, 48.5 ± 3.40 MPa and 41.6 ± 3.91 MPa respectively. Cubes (100 x 100 x 100 mm³) were used for the accelerated carbonation tests. Cubes (40 x 40 x 40 mm³) for capillary water suction experiments were cut from mortar slabs (900 x 600 x 40 mm³). Cylindrical specimens were drilled out of mortar slabs for chloride migration (height (H)=50 mm and diameter (Ø)=100 mm), and for water vapour diffusion tests (H=25 mm and Ø =80 mm). For each mixture, slabs were cast and cured for one day at 20°C and at a relative humidity (R.H.) of about 90 %. The specimens were demolded after 24 h and then stored in water for 27 days prior to the drilling of the specimens. Afterwards the specimens were stored under humid atmosphere (60 % R.H., 20°C) until application of the surface treatment at the age of 1-2 months.

2.2 Micro-organisms and growth conditions

Bacillus sphaericus LMG 225 57 (Belgian co-ordinated collections of micro-organisms, Ghent) was used for this study. Selection of this strain was based upon earlier work by our research group. This strain showed a high urease activity, a continuous formation of dense calcium carbonate crystals and a very negative zeta-potential [8, 9]. Liquid culture media consisted of 3 g/L nutrient broth (Oxoid N.V., Drongen, Belgium), 2.12 g/L NaHCO3, 10 g/L ammonium chloride and 10 g/L urea (VWR International, Leuven, Belgium). Liquid media were sterilized by autoclaving for 20 min. at 120°C. Cultures were incubated at 28°C on a shaker at 100 rpm for 24 h.

2.3 Treatment procedure

The biodeposition treatment was applied on the side opposite to the troweled face. Mortar specimens were immersed for 24 hours (15±5 mm depth) in a one day old stock culture of Bacillus sphaericus (ca. 107 cfu/mL). After this inoculation, specimens were wiped with a paper towel to remove some bacteria from the surface. In this way ureolytic activity primarily resulted from bacteria inside the specimens. Following this wiping, specimens were immersed (15±5 mm depth) in solutions of varying composition in order to investigate the effects of the external calcium source: without Ca, with $CaCl_2$ or with calcium acetate (CaAc). The specimens were removed from the solution after 3 days.

2.4 Scanning electron micrograph (SEM) examination

Cylinders (H= 10 mm, Ø= 6 mm) were drilled out of mortar cubes and treated with bacteria as described above. Samples were examined without preparation and coating of the samples. SEM micrographs were obtained using a FEI Nova 600 Nanolab Dual-Beam FIB.

2.5 Transport processes

2.5.1 Water absorption

To determine the increase in resistance towards water penetration a sorptivity test, based on the RILEM 25 PEM (II-6), was carried out. The mortar specimens (triplicate) were coated at the four edges adjacent to the treated side, to ensure unidirectional absorption through the treated side. After coating, the test cubes were dried at 45°C in a ventilated oven, establishing a mass equilibrium of less than 0.1% between two measurements at 24 hour intervals. The specimens were then exposed, to 10±1 mm of water, with the treated side facing downwards (water level about 2 mm above the base of the specimen). This was done in an atmosphere of 20°C and R.H. of 60%. At regular time intervals (15 min, 30 min; 1h, 1.5h, 3 h, 5 h, 8 h, 24 h, 72 h, 96 h, 120 h, 144 h and 168 h) the specimens were removed from the water and weighed, after drying the surface with a wet towel. Immediately after the measurement the test specimens were submerged again. The sorptivity coefficient, k [$cm.s^{-1/2}$], was obtained by using the following expression:

$$\frac{Q}{A} = k\sqrt{t} \qquad (1)$$

where Q is the amount of water absorbed [cm^3]; A is the cross section of the specimen that was in contact with water [cm^2]; t is the time [s], Q/A was plotted against the square root of time, then k was calculated from the slope of the linear relation between the former.

2.5.2 Water vapour diffusion

The influence of the surface treatment on water vapour diffusion was investigated by means of the wet cup method according the German Standard DIN 52615 [9]. The test cup was filled with a saturated solution of $NH_4H_2PO_4$.

A small air space was left below the test material resulting in a R.H. near 93% in the test cup, which is surrounded by the 60% relative humidity environment in the chamber (20°C). In this procedure water vapour is driven from the cup into the chamber through the specimen and the cup loses mass. Determination of the weight loss of the cups was done at weekly intervals. From the drying curve the water vapour diffusion coefficient, D [$m^2.s^{-1}$], can be calculated as follows:

$$D = J.\frac{R.T}{M_w}.\frac{L}{p_1 - p_2} \qquad (2)$$

where J is defined as the weight loss per unit of time and surface area [$g/s.m^2$], R is the universal gas constant [J/mol.K], T is the temperature [K], M_w is the molecular weight of water [g/mol], L= thickness of the specimen, p_1 and p_2 are the water vapour pressures at the side with high and respectively low R.H. [N/m^2]. Prior to testing, specimens were placed in an atmosphere of 20°C and a R.H. of around 90% until a 0.1% mass difference between two measurements during a 24 hour interval was obtained. Specimens were positioned with the surface treatment towards the side of high relative humidity.

2.6 Degradation processes

2.6.1 Accelerated carbonation test

The accelerated carbonation tests were performed in a CO_2-closet at a temperature of 20±3 °C, a R.H. of 70±10 % and a CO_2 concentration of 10 %. Before the test, the mortar cubes were dried at 60°C establishing a mass equilibrium of less than 0.1% between two measurements during a 24 hour interval. The following day the surface treatment was applied on the side opposite to the troweled face. The remaining sides were coated in order to prevent the penetration of CO_2. Then, the specimens were stored in an atmosphere of 20°C and R.H. of 60% for 14 days prior to start of the accelerated carbonation. A slice (10 mm) perpendicular to the treated surface was cut off and sprayed with phenolphthalein solution for determination of the carbonation depth. The remaining specimen was coated and put back in the CO_2-closet. Carbonation depth was determined after 2, 4 and 6 weeks. Resistance towards carbonation is expressed as the carbonation rate constant (K) [$mm.s^{-1/2}$], obtained as follows:

$$x = K.\sqrt{t} \qquad (4)$$

where x is the mean carbonation depth [mm] after a certain time [years] [10].

2.6.2 Accelerated chloride migration

The resistance towards chloride diffusion was investigated by means of the CTH rapid test according to the NT Build 492 Nordtest method. In brief, an external electrical potential was applied axially across the specimen. This forced the chloride ions outside to migrate into the specimen. After a certain test duration the specimen was axially split and a silver nitrate solution was sprayed on the freshly split sections. The chloride migration penetration depth was measured from the visible white silver chloride precipitation.

Results are expressed as the non-steady-state migration coefficient:

$$D_{nssm} = \frac{0.0239.(273+T).L}{(U-2).t}.\left(x_d - 0.0238\sqrt{\frac{(273+T).L.x_d}{U-2}}\right) \quad (5)$$

where D_{nssm} is the non-steady-state migration coefficient [m²/s], U is the absolute value of applied voltage [V], T is the average value of the initial and final temperatures in the anolyte solution [K], L is the thickness of the specimen [m] and x_d is the average value of the penetration depth [m]. [11, 12]

2.7 Statistical analysis

Experiments were performed in triplicate. Error bars on graphs show the standard error. After univariate analysis, grouping of treatments based on significant differences in mean values was done according Student Newman Keuls or Dunnet T3 tests (0.05 level of confidence), depending on homoscedasticity results of the Levene test.

3 Results

3.1 SEM examination

Scanning electron micrographs of the surface of an untreated mortar sample (Fig. 1a) and a sample treated with bacteria and calcium acetate (Fig. 1b-d) show distinct differences. The treatment of mortar cubes with bacteria and a calcium source resulted in the presence of crystalline deposits on the surface. The majority of the carbonate deposits was present as calcite crystals as was confirmed by XRD analyses. Besides calcite, small amounts of vaterite were present. On closer observation, spherical deposits (presumably vaterite) of around 30 µm were visible within a dense matrix of irregular crystalline deposits (Fig. 1c). Rhombohedral crystals, characteristic for calcite, are present in the middle of the micrograph (Fig. 1d). The rod shaped holes inside the crystals show the space that was occupied with the bacteria.

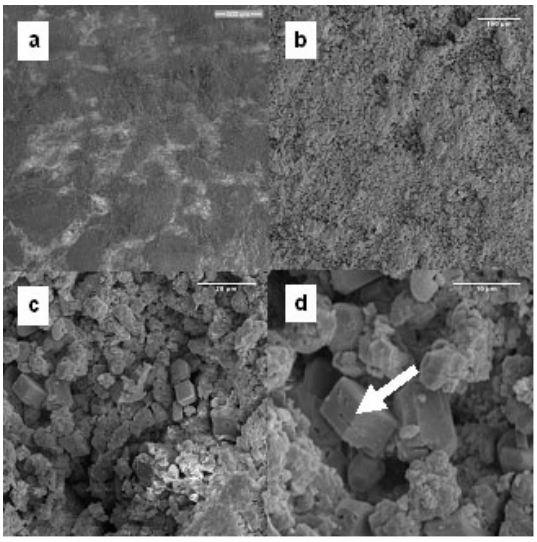

Figure 1: Scanning electron micrographs showing the surface of (a) an untreated mortar cube and (b) a cube treated with bacteria and calcium chloride; (c) Magnification of the area in (b); (d) Enlarged image of a section in (c). Rod shaped holes (see arrow) indicate bacterial mediation of crystallization

3.2 Transport processes

3.2.1 Water absorption

Fig. 2 shows the influence of the surface treatment on the water absorption rate for mortar cubes with a w/c 0.6. Over a period of 168 hours the cubes treated with bacteria and calcium chloride absorbed nearly five times less water than the control cubes. The presence of only bacteria resulted in a significant decrease of the water uptake compared to untreated specimens (a reduction of 45%, 43% and 24% with increasing w/c). When a calcium source was added to the medium an additional significant decrease of the water absorption coefficient was noticed. The decline in water absorption, compared to the untreated cubes, amounted to 85%, 90% and 75% respectively for the mortar specimens with w/c 0.5, w/c 0.6 and w/c 0.7.

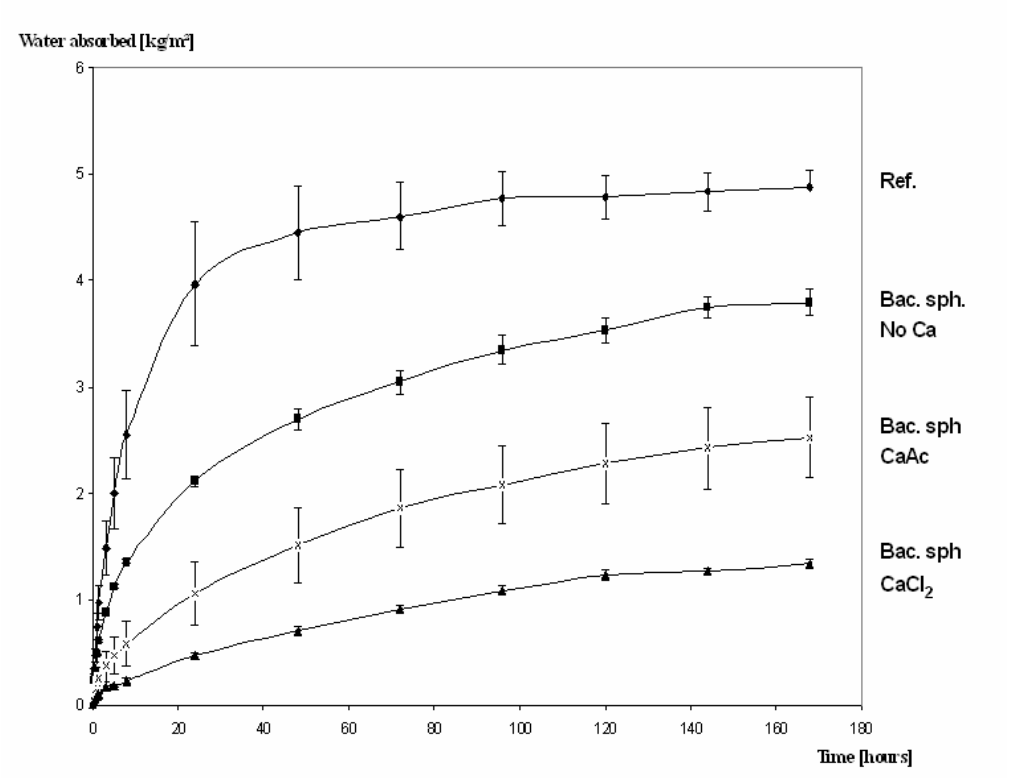

Figure 2: The influence of the surface treatment on the rate of water absorption versus time for mortar cubes (w/c 0.6)

3.2.2 Water vapour diffusion

The effect of the water-cement ratio and the surface treatment on the water vapour diffusion of mortar is presented in Fig. 3. Specimens with a higher w/c showed a larger water vapour diffusion coefficient. The presence of bacteria resulted in a decrease of the diffusion coefficient. Addition of a calcium source led to an additional decrease of the diffusion coefficient. Differences between untreated specimens and specimens treated with bacteria were however not significant.

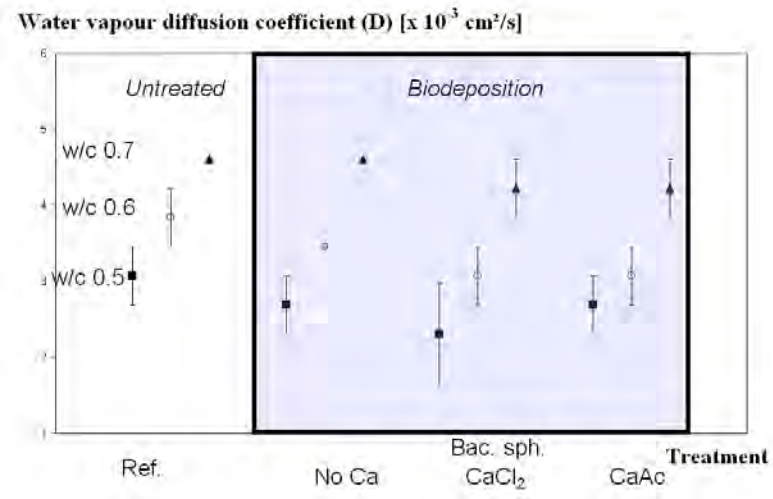

Figure 3: Watour vapour diffusion coefficients, D, for different mortars (w/c 0.5: ■, w/c 0.6: ● and w/c 0.7: ▲) with different surface treatments

3.3 Degradation processes

3.3.1 Carbonation

The effects of the water-cement ratio and the surface treatment on the resistance of mortar cubes towards carbonation are summarized in Fig. 4. The carbonation rate increased with increasing w/c. Significant differences in carbonation depth between treated and untreated specimens were already noticeable after 2 weeks of accelerated carbonation (results not shown). The addition of bacterial biomass resulted in a significant smaller carbonation rate compared to untreated cubes. The effect of this addition improved with increasing w/c, resulting in a decrease of K about 24 %, 30 % and 37% respectively. The combination of the biomass and a calcium source resulted in an additional drop of the carbonation rate; a decrease of 53%, 58% and 44% (compared to untreated specimens) was noticed with increasing w/c. There were no considerable distinctions in carbonation rates between treatments with a different calcium source (acetate or chloride).

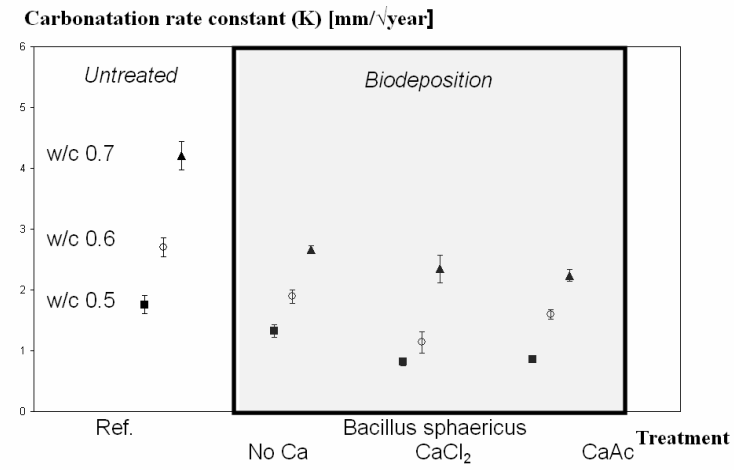

Figure 4: Carbonation rate constants, K, for different mortars (w/c 0.5: ■, w/c 0.6: ● and w/c 0.7: ▲) with different surface treatments

3.3.2 Chloride migration coefficient

The resistance of mortar specimens towards the diffusion of chlorides expressed as the chloride migration coefficient is shown in Fig. 5.
For untreated specimens the value of this coefficient increased with increasing water-cement ratio. With exception of the biomass only treatment, there was an increased reduction of D_{nssm} with increasing w/c. The addition of bacterial biomass resulted in an increased resistance towards the penetration of chlorides compared to untreated cubes. The results were most pronounced for the less porous specimens (w/c 0.5), which obtained a 19 % decrease of the chloride migration coefficient. The combination of the biomass and a calcium source resulted in significant lower chloride migration coefficients compared to untreated specimens (a decrease of 25%, 25% and 30% with increasing w/c). There were no considerable distinctions between treatments with a different calcium source (acetate or chloride).

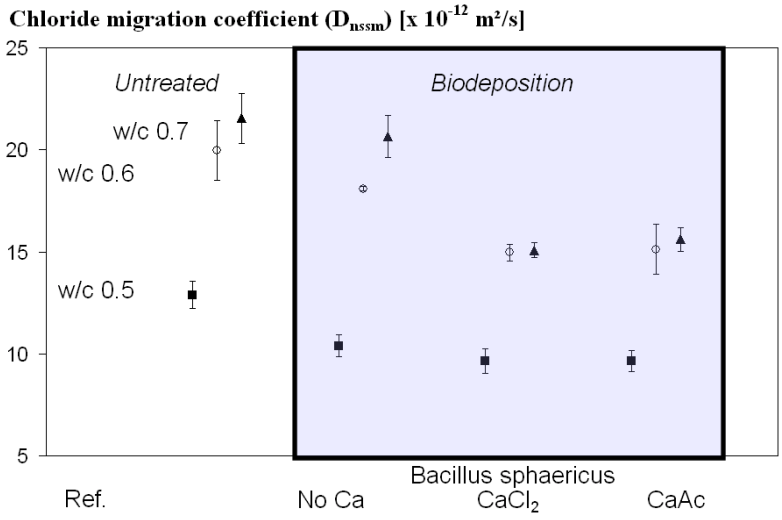

Figure 5: Chloride migration coefficients, D_{nssm}, for different mortars (w/c 0.5: ■, w/c 0.6: ● and w/c 0.7: ▲) with different surface treatments

4 Discussion

In this research, it was shown that bacterial carbonate precipitation improved the resistance of mortar specimens to degradation processes. The deposition of a layer of calcium carbonate crystals on the surface resulted in a decrease of the permeation properties. As a consequence, the ingress of harmful substances was limited. From the above, it is clear that the presence of a layer of carbonate crystals on the surface has the potential to improve the resistance of cementitious materials towards degradation processes.
The decrease in permeability of mortar specimens treated with bacteria could be seen from the water absorption and water vapour diffusion experiments. As a result of the biodeposition, specimens with a w/c 0.6 showed significantly less water absorption than untreated specimens with a w/c 0.5. However, specimens with a w/c 0.7 on which the biodeposition treatment was applied showed a slightly higher water absorption coefficient than the latter. The higher water absorption coefficient of the most porous specimens with the biodeposition treatment could be due to an incomplete coating of the surface. Repeated application of bacteria and a calcium source could result in an additional decrease of the water absorption. Nemati and Voordouw noticed an additional decrease of the permeability of sandstone cores after injecting $CaCO_3$ forming reactants for a second time [13].
The permeability of mortar specimens to water vapour was not significantly altered by the application of the biodeposition method. Sufficient permeability of surface treated concrete to water vapour is necessary to allow the substrate and any reinforcement it contains to remain substantially dry.
The biodeposition treatment resulted in an increased resistance towards carbonation. The rate of carbonation and the performance of the surface treatment were correlated to the water-cement ratio. Several authors have demonstrated an increase in the carbonation rate in concrete with increasing water-cement ratio. Carbonation was shown to be related to the nature of the pores, with larger pores giving rise to higher carbonation depths [10]. Basheer *et al.* reported that for film forming coatings and sealants in order to be effective against carbonation, the thickness of the treatment should be at least 200 µm [14]. Preliminary observations from SEM and µCT analyses indicated that the thickness of the calcite layer is at least between 50 and 100 µm.

The presence of the biodeposition treatment improved the resistance towards chloride penetration. In this research, resistance towards chloride penetration was measured with the use of an accelerated migration test. Published work on the chloride resistance of surface treated concrete however, is mainly based on the results of diffusion tests [14, 15].
The long duration of this method however, has encouraged some researchers to investigate the use of more rapid tests [16]. Buenfeld and Zhang commented on the use of diffusion coefficients obtained for surface treated concrete from non-steady state immersion tests. They proposed the use of a pseudo diffusion coefficient which accounted for the diffusion resistance of the surface treatment layer. This pseudo diffusion coefficient is lower than the true diffusion coefficient calculated from the untreated control substrate concrete [15]. Similarly the results from the CTH tests in the current research could be expressed as pseudo migration coefficients, since the calculation of the non steady state migration coefficient [16] is also based upon Fick's second law with fixed boundary concentration.

A lot of research on biodeposition has been conducted with $CaCl_2$ as the calcium source [2, 17, 18]. As chloride ions are detrimental for the reinforcement, the use of calcium acetate as an alternative calcium source was investigated in our research.

Results show that a similar protective performance was obtained with the biodeposition treatment in the presence of calcium chloride or calcium acetate. In order to avoid possible adverse effects of the calcium chloride on concrete, future research will be done by using calcium acetate as calcium source.
As calcium carbonate is solubilised in acidic environments, there is a need to investigate the effect of acidic rain on the durability of the biodeposition treatment. Bacterially induced calcite crystals however, are assumed to be more resistant to dissolution since it has been experimentally demonstrated that biologically deposited calcite is less soluble than inorganically precipitated calcite [19].

5 Conclusions

The current study demonstrated that the biodeposition treatment resulted in an increased resistance of mortar specimens towards degradation processes. The results from this research tend to confirm the interrelationship that exists between transport and degradation mechanisms occurring in concrete. The transport mechanisms in concrete are influenced by the surface treatment with the ureolytic bacteria and a calcium salt. The presence of a layer of calcium carbonate and microbial biomass resulted in a decrease of the permeation properties of cementitious materials. As a result, an increased resistance towards carbonation and chloride migration was noticed. Further research however is warranted to investigate the impregnation depth and the durability of the treatment under environmental conditions.

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